About the role of the apolipoprotein E4 polymorphism in dementia: does genetic testing have a place in the assessment of Alzheimer's disease?

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ABBREVIATIONS

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; apoE, apolipoprotein E protein; APOE, apolipoprotein E gene; APP, amyloid precursor protein; Arg, arginine; Aβ, amyloid-β peptide; BBB, blood-brain barrier; CNS, central nervous system; Glu, glutamic acid; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; LOAD, late-onset Alzheimer's disease; MS, multiple sclerosis; OR, odds ratio; PDD, Parkinson's disease dementia; REVEAL, The Risk Evaluation and Education for Alzheimer's disease; tau, microtubule-associated protein tau; TR, targeted replacement; VLDL, very-low-density lipoprotein.

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ABSTRACT

The ε4 allele of the apolipoprotein E (APOE) gene is a strong risk factor for developing late-onset Alzheimer's disease (LOAD), accounting for about half of the genetic component of the disease risk. Recent investigations into the mechanisms by which APOE4 contributes to the pathology of LOAD is offering new perspectives on how the disease may be treated in the future, and even though further research is needed before any such drugs will be made available, we may see the emergence of strategies for genotype-specific prevention and therapy. Current efforts are focused on how apolipoprotein E isoforms affect different aspects of the disease process, including the production and clearance of amyloid-β peptide, amyloid neurotoxicity, tangle formation, cholesterol homeostasis, and synaptic plasticity and repair. Since carrier status at present offers no benefits in terms of more accurate prognosis or better therapy, genotyping is discouraged for all other purposes than research, and there are concerns about the mental health of susceptible individuals receiving discouraging results. There are ethnical and geographical variations in the distribution of the APOE4 allele and in its association with LOAD. This, along with the fact that the APOE4 allele is neither sufficient nor necessary for the development of LOAD, prompts us to think that we are only beginning to get a grasp on how to counteract this leading cause of dementia, in a world where demographics are presenting us with more care demanding elderly than ever before.

INTRODUCTION

On the 14th of April 2003, a statement was released by the International Human Genome Sequencing Consortium, announcing the successful completion of the Human Genome Project. This entailed that about 99 % of the human genome's gene-containing regions had been sequenced to an accuracy of 99.99 % (1). However, inherited genetic variance has a critical role in human disease, and the Human Genome Project shed little light on this variance, having been completed by sequencing the DNA of just a few anonymous donors (2). The HapMap project was subsequently launched in order to map the millions of single nucleotide polymorphisms which make each individual unique (3). Despite the ever-accelerating pace of biomedical research, and the fast increasing amount of knowledge we have of the human genome, root causes of human diseases are still largely unknown. Genetic inheritance seems to play a vital role in nearly all of them, and a lot of emphasis is now being placed on making the connections. Investigations into how individual genes contribute to pathology may allow earlier diagnosis, better preventive measures, and maybe even curative treatments.

The term polymorphism, originally coined by E B Ford as early as 1940, during his investigations into human blood groups, was originally used in biology to describe two or more different phenotypes occurring at the same time in the same species (4). The definition most used in current genetics is that *a polymorphism is a genetic variation that occurs with at least 1 % frequency in a population* (5). In this paper, I intend to describe one such polymorphism, namely that of the apolipoprotein E, and investigate its effects on neurological disease in humans.

Apolipoprotein E

Apolipoproteins were discovered in the early 1960s, as scientists were beginning to dissect human lipoproteins, providing the possibility to isolate smaller components. The name itself was first suggested by J L Oncley, a professor of bio-chemistry at the University of Michigan (6). The prefix apo, meaning *separate* or *detached*, suggests that an apolipoprotein is the protein moiety in its lipid-free form (7).

Apolipoprotein E (apoE), initially termed the arginine-rich apoprotein, was first identified as a constituent of very-low-density lipoprotein (VLDL) in 1973, by Shore and Shore (8). It was subsequently demonstrated that apoE is present not only in VLDL, but in chylomicrons, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). The function of apoE became clear shortly after its discovery, and by 1982 numerous experiments had shown that it was important in the catabolism of lipoproteins, both in hepatic and extrahepatic tissues (9). The first clue to the possible detrimental effects of one of its polymorphisms was also presented as early as in 1973, when a strong association was shown between apoE and type III hyperlipoproteinemia (10).

The polymorphic nature of the apolipoprotein, however, was not clearly established until the early 1980s (11;12). The nomenclature designating the three major isoproteins as apoE2, apoE3, and apoE4, was set in 1982, along with the classification of the alleles that produced them (APOE2, APOE3, APOE4), and the certification of the fact that they belong to a single gene locus, on chromosome 19 (13;14). Simple genetics then dictated that six different phenotypes existed, three homozygous (apoE2/2, E3/3, and E4/4) and three heterozygous (apoE2/3, E2/4, and E3/4) (14). Subsequent population studies revealed that the most common phenotype is apoE3/3 and that the most common allele is APOE3, leaving apoE3 to be considered the parent protein, while E2 and E4 were considered the variants (15;16). Apolipoprotein E2 came to be most commonly associated with type III hyperlipoproteinemia, and was found to be defective in receptor binding (17), while apoE4 displayed normal receptor binding, but was associated with elevated plasma cholesterol and LDL, thus predisposing carriers to coronary artery disease (18).

By 1988, it had been discovered that apoE had more functions than previously assumed. A major function was indeed that of transporting lipids from sites of synthesis or absorption to sites of utilization, but equally important was deemed its role of redistributing lipid within a tissue during injury and repair. ApoE was found to be synthesized in various cell types, such as hepatocytes, macrophages, astrocytes and smooth muscle cells. The large accumulation of apoE in response to nerve injury suggested the possibility of involvement in processes like neurite membrane biosynthesis and myelin formation. ApoE was also suspected of playing a role in smooth muscle cell proliferation and differentiation (8).

An unexpected revelation

Even though the perceived role of apolipoprotein E had expanded during the late 1980s, focus remained on apoE4's involvement in elevated levels of total and LDL-cholesterol, and the elevated risk of coronary artery disease for its carriers (19). Therefore, in 1993, it was considered a major breakthrough when Warren J Strittmatter, Allen D Roses, and colleagues, published an article that suggested *A functional role of the apolipoprotein E-E4 isoform in the pathogenesis of late-onset familial Alzheimer disease*. They had found that apolipoprotein E was immunochemically localized to the senile plaques, vascular amyloid, and neurofibrillary tangles of Alzheimer's disease (AD). Furthermore, their investigation into the frequency of apoE alleles in Alzheimer patients and controls showed a highly significant association between apoE4 alleles and late-onset familial Alzheimer's disease (LOAD) (20).

The following years, a lot of material was published regarding the association between apoE4 and certain types of neurological disease. In 1995, a much cited review by Strittmatter and

Roses, *Apolipoprotein E and Alzheimer disease*, summarized the early years of this research. In it, they stated with little ambiguity that inheritance of specific apoE alleles, in large part determines the risk and mean age of onset of late-onset familial and sporadic Alzheimer disease. They had evidence of isoform-specific differences in the binding of apoE to the microtubule-associated tau-protein, which constitutes most of the neurofibrillary tangles (FIG 1A) in AD, and to amyloid-β peptide, which is a major component in neuritic plaque (FIG 1B) (21). A year later, another review was published under the same name. The second review was much more comprehensive, and provided even more certainty that previous assumptions had been justified. Little groundbreaking information was given, however, it was stated more clearly than before that the APOE4 allele indeed increased the probability of disease at an earlier age, and that the APOE2 and APOE3 alleles decreased the probability of disease and increased the age of onset (22).

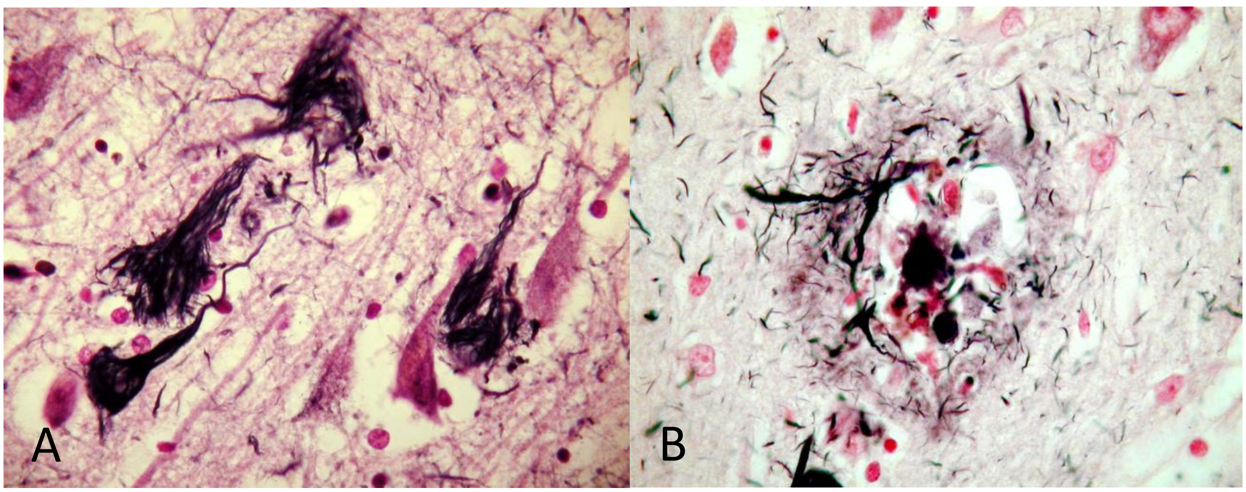

Figure 1 | **Gallyas staining, 40x objective. A – Neurofibrillary tangles. B – Neuritic plaque.** Oslo University Hospital Ulleval.

Association with other diseases in the 20th century

Already in their breakthrough article of 1993, Strittmatter and Roses had noted that apoE synthesis increased following injury, and was similarly increased in several chronic neurodegenerative disorders, not just in Alzheimer's disease (20). In 1991, scientists had found high levels of apoE in the amyloid plaques of Creutzfeldt-Jakob disease (22). Over the following decade, apoE4 was linked to earlier onset and more severe progression of diabetic neuropathy (23), while studies concerning human immunodeficiency virus (HIV)-related neuropathy, motor neuron disease and amyotrophic lateral sclerosis (ALS), were too methodically weak to provide any solid leads (24). In Guam, two studies regarding the ALS/parkinsonism/dementia complex, found significantly lower frequencies of apoE2 in patients compared to controls, suggesting a protective function of apoE2 (25;26). Concerning cerebral amyloid angiopathy, apoE4 was shown to be a risk factor, with or without pathological evidence of AD (27;28) Two individual studies demonstrated elevated APOE4 frequencies among patients with Lewy body dementia (29;30), and among the AD-prone patients with Down syndrome, several studies had shown an earlier age of dementia onset for APOE4 carriers, and significant protection against dementia from APOE2 (31).

ApoE4 was perceived to influence all neurodegenerative disorders associated with the deposition of amyloid-β peptide (Aβ), while apoE variation was not shown to have any effect on the prevalence of disorders such as Parkinson’s disease without dementia, progressive supranuclear palsy, ALS, multiple sclerosis (MS), Huntington’s disease, depression or schizophrenia (32). There was no consistent association between apoE4 and Creutzfeldt-Jakob disease (33) or HIV-associated dementia (34). Thus, apoE4 remained firmly linked only to neurological disorders involving the deposition of Aβ (32).

A review from 2000 found a moderately increased risk of coronary artery disease among apoE4 carriers (32), and a 1999 meta-analysis concerning ischemic cerebrovascular disease found a moderately increased risk for patients with apoE4 as well (35). Further elucidating its role in cholesterol metabolism, apoE4 was shown to increase the cholesterol content of gallstones, and also to increase the prevalence of gallstones in women (36).

Interestingly, there was shown no connection between the neurological and cardiovascular risk induced by apoE4. A large epidemiological study in New York found no consistent relationship between apoE genotype, plasma cholesterol and AD among its elderly (37), and a large case-control study in Rotterdam concluded that apoE4’s effect on dementia was not through atherosclerosis (38).

Regarding diseases neither neurological nor cardiovascular, one longitudinal study involving 1750 women over the age of 65, showed an increased rate of bone loss for E4 carriers, and an increased frequency of hip fractures, even after correcting for age, mental status and bone mineral density (39). ApoE was shown to be implicated in immunoresponses against various infectious agents, but no certain relation to phenotype variants had been shown by 2000 (40).

Finally, from the Strittmatter/Roses breakthrough in 1993, until 2000, the association between the APOE4 allele and Alzheimer’s disease was one of the most repeated findings in medical genetics (32).

Geographical distribution

The APOE gene is structurally similar to some of the APOA and APOC genes, and is thought to have diverged from the APOA-I and APOA-IV genes some 420 million years ago (41). Research to date indicates that APOE4 is the ancestral allele of E2 and E3. Almost all animal APOE more closely resembles the human APOE4, including that of all the great apes (42). Furthermore, Fullerton et al. identified sequence haplotype[1] variation for the whole APOE locus among an ethnically diverse sample, and inferred from the pattern of haplotype relationships that APOE2 and E3 are derived from the genetically older APOE4 (43).

It may seem strange that the now deleterious APOE4 allele was the origin of the apparently more beneficial other two. However, the APOE4 may have been rendered causal of diseases like coronary artery disease and Alzheimer’s disease, by environmental conditions such as a longer lifespan and the western diet. This idea is supported by the fact that APOE4 is not associated with either disorder in sub-Saharan Africans, but it is associated with both of them in African Americans (44).

Globally, the APOE locus shows substantial allelic variation. APOE2 ranges from 0-20 %, APOE3 ranges from 60-90 %, and APOE4 ranges from 10-20 % (44), with a few exceptions (45). The APOE3 allele is the most frequent in all human groups, especially in populations where an agricultural economy was established early, such as in the Mediterranean basin, where it ranges from 0.849 to 0.898. The frequency of the APOE4 allele remains higher in indigenous populations where an economy of foraging still exists, or food supply is scarce and sporadically available, e.g. among aborigines, pygmies or Native Americans. Interestingly, the APOE4 allele is absent in the tribal population of Koch, India (45). The APOE2 frequency fluctuates with no apparent trend, and is absent in Native Americans (44). Figure 2 shows APOE4 distribution worldwide, below.

Possible explanations for the geographical distribution of the alleles exist. APOE4 carriers are thought to be more responsive to dietary fats, and to have better absorption of lipids and fat-soluble vitamins. This presents an advantage when food sources are scarce, and may be the reason why it is more common in populations that up until recently have been hunter-gatherers (44). Although APOE4 has been found to be causative in many diseases, it still occurs at high frequencies in some populations. Pre-historical selective benefits probably outweigh its disease cost (45). In long-established agricultural communities, e.g. southern Europe, Southeast Asia and Central America, it is possible that APOE3 was selectively favored once populations reached the

[1] Alleles located in close proximity on the same chromosome that, as a result, are inherited together. In a population, the genome is partitioned into haplotype blocks of varying length depending on the strength of the linkage disequilibrium between the alleles, and the different combinations of alleles that are located in these blocks are called haplotype clades or haplotype alleles.

critical size needed to sustain certain viral infections, from which it provided better protection than APOE4 (40), or that the late cognitive impairment produced by APOE4 was a disadvantage

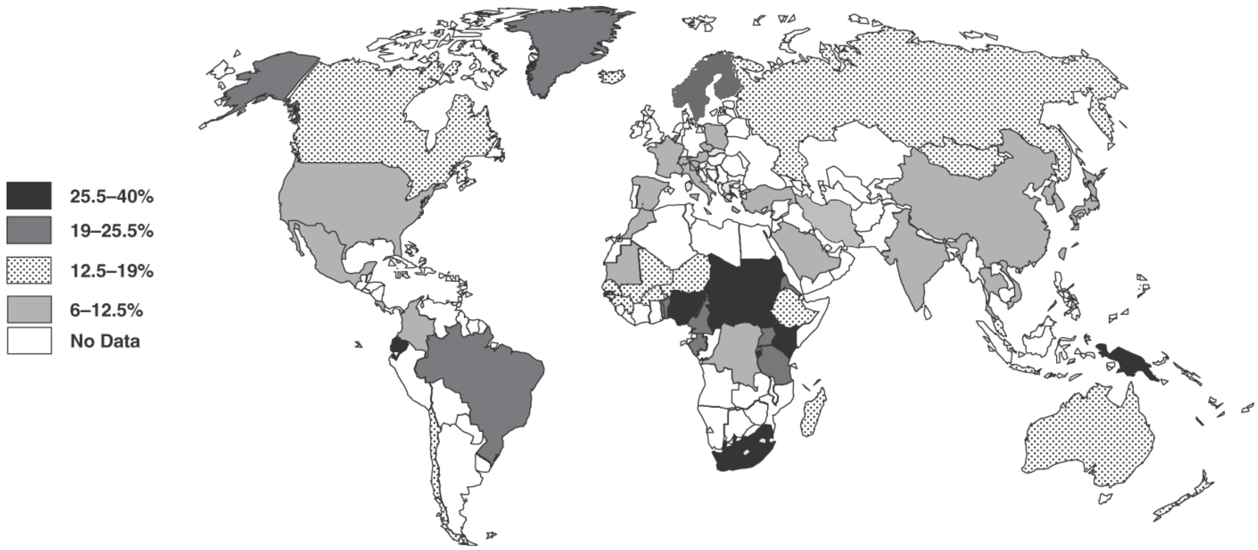

Figure 2 | **Worldwide distribution of the APOE4 allele.** Figure is from Singh et al. 2006, with corrections made according to Kumar et al. 2002 and Raygani et al. 2005.

when family elders needed to pass on more complex information, thus providing worse conditions for their young (46). Another factor may be that the APOE allele influences reproductive efficiency, one study has shown that male homozygous APOE3 carriers have more children than carriers of APOE2 and APOE4 (47).

In Europe, there is a significant south to north increasing cline of the APOE4 allele, and the APOE4 frequency worldwide is generally higher in dark-skinned people(45). This may be explained by APOE4's association with better intestinal absorption of vitamin D, more sorely needed where sunlight is scarce or in dark-skinned people, thus influencing natural selection (45).

Finally, factors such as genetic drift, local selection, and isolation by distance cannot be excluded when attempting to explain the variance of APOE genotypes around the world. In any case, the existing population diversity clearly indicates that the APOE polymorphism is a useful anthropogenic marker for population genetic studies (45).

PROBLEM

The different alleles of the apolipoprotein E polymorphism have a large impact on human health. In fact, one could claim that since this single gene variation has had a major influence on human evolution, and has a central role in the pathogenesis of Alzheimer's disease, the variation in the APOE-gene is one of the most important polymorphisms within the science of genetics. Therefore, this paper is written with the intention of providing answers to the following questions:

What impact does the apolipoprotein E4 have on the risk of developing Alzheimer's disease?

What are some of the pathological mechanisms by which the apolipoprotein E4 contributes to the development of Alzheimer's disease?

Does knowledge of these mechanisms reveal any new insight into the possible treatment of Alzheimer's disease?

Does apolipoprotein E genotyping offer any benefit to patients of Alzheimer's disease, in terms of prognosis, risk reduction or treatment?

ASSESSMENT OF DEMENTIA
In most patients

• Imaging of head (CT/MRI)
• Blood test
    ○ Full blood count, ESR
    ○ Urea, electrolytes, glucose
    ○ Calcium, liver function tests
    ○ Thyroid function tests
    ○ Vitamin B_{12}
    ○ Venereal Disease Reference Lab test
    ○ ANA, anti-dsDNA
• Chest radiograph
• EEG

In selected patients
• Lumbar puncture • HIV serology • Brain biopsy

Table 1 | **From Davidson's Principles and Practice of Medicine 2002.** Should APOE genotyping appear somewhere in this box?

METHODS

This non-systematic review has been completed by 6th-year medical student at the University of Oslo, Einar Aronsen, under the supervision of Professor Jan Maehlen, M.D., Ph.D. Searches were made on the Cochrane libraries, PubMed, and MEDLINE. With the exception of the Lehninger Principles of Biochemistry (7) and Davidson's Principles and Practice of Medicine (48), no textbooks were used. Some online resources have been quoted: the official websites of The International Human Genome Sequencing Consortium, the AlzGene database, and the Norsk Elektronisk Legehandbok (the Norwegian Electronic Doctor's Handbook).

Search-words were: *Alzheimer, apoE, apoE4, apolipoprotein E, apolipoprotein E4, atherosclerosis, cardiovascular disease, distribution, genetic testing, history, polymorphism,* and combinations of these using *AND* and *NOT*. No further limitations were placed on searches. Additional searches were made using authors and titles from references listed in articles produced by primary searches.

ALZHEIMER’S DISEASE

The role of the apolipoprotein E4 polymorphism

Alzheimer’s disease (AD) in a given patient can be categorized as either early-onset AD, which is inherited in a classic autosomal dominant manner[2], or late-onset AD (LOAD), for which the pattern of inheritance is much more complex. Early-onset AD is quite rare and accounts for less than 2 % of cases (49). All mutations currently known to cause early-onset AD (i.e. onset before the age of 60) are located either in the amyloid precursor protein (APP)-gene, or in the presenilin protein genes 1 and 2. Mutations in these genes alter the normal processing of APP to the amyloid-β peptide (Aβ), the deposition of which is hallmark to all forms of AD, but especially to the early-onset form (5). People inheriting one of these autosomally dominant mutations will develop AD unless they die prematurely from other causes (49).

Apolipoprotein E (apoE indicates the protein and APOE indicates the gene) is a plasma protein involved in the transport of cholesterol, which is encoded by a gene on chromosome 19 (50). Since it was first associated with AD in 1993 (20), confirmations in numerous ethnic populations have established APOE genotype as the most important biological marker for susceptibility for AD, accounting for 45 % to 60 % of it’s genetic component (51). This bears more relevance for investigations into LOAD, but the disproportionate representation of APOE4 is also present in patients with early-onset AD (52). Interestingly, the APOE4 allele is neither necessary nor sufficient to cause AD, as at least a third of patients are not carriers of the APOE4 allele and almost 50 % of homozygous carriers that survive until the age of 80 do not develop AD (49).

A meta-analysis by Farrer et al., published in 1997, was the first to present a comprehensive overview of the distribution of APOE genotypes around the world, and its effects on AD (49). The most recent compilation of these data published on paper came in 2006 (45), and there is a publicly available database, called AlzGene, that attempts to keep up with the ever-more rapid flow of new investigations (5;53). It is interesting to note that new findings are almost completely in harmony with the meta-analysis from 1997, providing only minor adjustments based on more in-depth investigations into population minorities and some changes in methodology (5;54).

Heterozygous carriers of the APOE4 allele have an odds ratio[3] (OR) range of 2.2-4.4 for developing AD, using as reference (OR=1) homozygous carriers of the APOE3 allele. The OR

[2] A type of inheritance in which the phenotype of a trait is determined completely by one of two alleles on the non-sexchromosomes. There can be either one (heterozygous state) or two (homozygous state) copies of the dominant allele.

[3] A measure of effect size (for example, of risk effects). The OR measures the ratio of the odds of an event occurring in one group (for example, disease cases) to the odds of that same event occurring in another group (for example,

range of homozygous APOE4 carriers is 5.1-34.3. Conversely, the APOE2 allele offers some protection from the disease, being underrepresented in AD patients, although the collective evidence for this assumption is less comprehensive (49). The APOE genotype is also linked to age of onset, in such a fashion that carriers of the APOE4 allele tend to be younger when clinically diagnosed, compared to carriers of APOE2 and E3. Also, homozygous carriers of E4 have an earlier age of onset than heterozygous carriers (55). It is not clear whether APOE genotype has any effect on the rate of cognitive decline in AD (56). Although more than 500 other genes have been assessed as potential LOAD risk factors, none have been consistently proven to influence disease risk (5), and none show odds ratio effects even close to those of APOE4 (typically less than 1.5 compared to non-carriers) (57).

According to the AlzGene database, i.e. the most comprehensive and current meta-analysis of APOE genotype distribution, the APOE4 allele is present in 38 % of all Caucasian AD patients, whereas the number is 14 % for controls. Furthermore, 14.5 % of all Caucasian AD patients are homozygous for APOE4, and only 2 % of controls. These numbers come out roughly the same when making estimations concerning all races in total, but that is largely due to the fact that a large majority of studies completed, have been done with Caucasian subjects (58). In some populations, e.g. African Americans and Hispanics, the association between APOE4 and AD appears much more uncertain than among Caucasians. It remains unclear whether this is due to smaller samples and weaker methodology, or if it is related to differences in genetic and environmental background (59). The APOE4-AD association has been described as the strongest in studies completed with Japanese subjects (49).

Pathogenesis

Physiological function

The human apolipoprotein E is a 299 amino acid glycoprotein (60). It is expressed in several organs, with the highest expression in the liver, followed by the brain. Astrocytes, and to some extent microglia, express the majority of apoE in the brain (61). Neurons may also exhibit some apoE expression, but only under certain conditions (e.g. injury), and at much lover levels than astrocytes and microglia (62). ApoE functions as a ligand in receptor-mediated endocytosis of lipoprotein particles. In plasma, apoE proteins are present on lipoproteins in association with other apolipoproteins, whereas in the brain apoE and two other apolipoproteins, apoJ and apoA-1, are predominantly present on distinct high-density-like lipoprotein particles (63). The predominant apolipoprotein of high-density lipoprotein (HDL) in the central nervous system

healthy controls). An OR of 2 indicates that carriers of a certain risk factor are at twice the risk of developing the disease as non-carriers; an OR of 0.5 indicates that the risk in carriers is only half that in non-carriers.

(CNS) is apoE (64). After receptor-mediated endocytosis of apoE-containing HDL-like particles by members of the low-density lipoprotein (LDL) receptor family, apoE may be either degraded or recycled back to the cell surface (65). Cholesterol released from apoE-containing lipoprotein particles is used to support synaptogenesis and synaptic maintenance (66). Whether this use of cholesterol occurs in the uninjured brain remains uncertain, as the brain of APOE knockout mice generally appears normal in the absence of injury (66).

The three isoforms apoE2, apoE3 and apoE4, differ at positions 112 and 158 (8). These single amino acid differences among the three apoE isoforms alter the protein's structure and influence its lipid association and receptor binding (67). ApoE4 has the amino acid arginine (Arg) at position 112, which affects the conformation of the side chain of Arg61, resulting in *domain interaction* between this Arg61 in the N-terminal domain and Glu255[4] in the C-terminal domain (57). This domain interaction is perceived to be central in causing most of apoE4's adverse effects (FIG 3) (67;68).

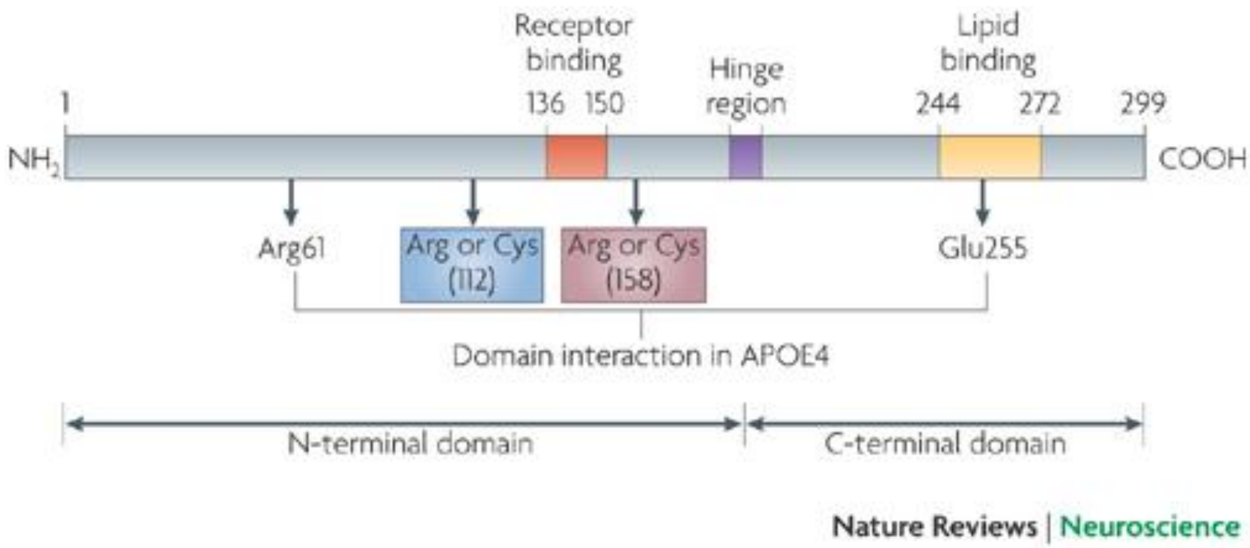

Figure 3 | **Schematic representation of human APOE.** The 299 amino acid human apolipoprotein E (APOE) contains two independently folded domains: an amino-terminal domain that includes the receptor-binding region and a carboxy-terminal domain that contains the major lipid-binding region. The residues that distinguish the APOE isoforms (112 and 158) are marked. APOE2 has Cys at both positions, APOE4 has Arg at both positions, and APOE3 has Cys at position 112 and Arg at position 158. The domain interaction between Arg61 and Glu255 in APOE4 is also indicated (Bu 2009).

[4] Glutamic acid.

Aβ production and clearance

The accumulation, oligomerization and subsequent deposition of amyloid-β in the brain are central events in the pathogenesis of AD (57), leading to the presence of amyloid plaque, which was immunoreactively shown to contain apoE as early as 1991 (69). Aβ level in the brain is the net balance of Aβ production and clearance, and accumulation could consequentially reflect overproduction, insufficient clearance, or both (57).

Several apoE receptors interact with the amyloid precursor protein (APP) and modulate its trafficking and processing to Aβ (70). ApoE isoforms differentially regulate APP processing to Aβ through these receptors, and apoE4 has been shown to increase Aβ production compared to apoE3 (71).

Aβ is actively cleared from the cerebrospinal fluid, at a rate of 8.3 % per hour (72). This happens through two major pathways. One is receptor-mediated clearance by cells in brain parenchyma, along the interstitial fluid drainage pathway or through the blood-brain barrier (BBB). The other is endopeptidase-mediated proteolytic degradation (57). ApoE receptors are likely to be involved in the former, as apoE is a well-characterized chaperone for Aβ (73). Since apoE3 binds to Aβ peptides with higher affinity than apoE4 (74), receptor-mediated clearance of Aβ is probably less effective for the apoE4 phenotype (75). Furthermore, Aβ binding to apoE4 redirects its clearance from the LRP1-receptor[5] to the VLDL-receptor, which has a slower clearance rate (76). In contrast, Aβ binding to apoE2 and E3 induces clearance at both receptor types (57). Even though receptor-mediated clearance in principle is an effective way of reducing Aβ levels in the brain, it is possible that clearance of Aβ into neurons can lead to intraneuronal Aβ accumulation, which is toxic (77).

As stated above, Aβ can also be removed from the brain by proteolytic degradation. ApoE promotes this degradation by way of the enzymes neprilysin and insulin-degrading enzyme, with apoE3 being more efficient than apoE4 (78).

Both post-mortem studies (79) and positron emission tomography imaging studies (80) have demonstrated an increased amyloid plaque load in brains of APOE4 carriers. Figure 4 shows the influence of the APOE isoforms on the major Aβ clearance pathways in the brain.

[5] Low-density lipoprotein receptor related protein 1.

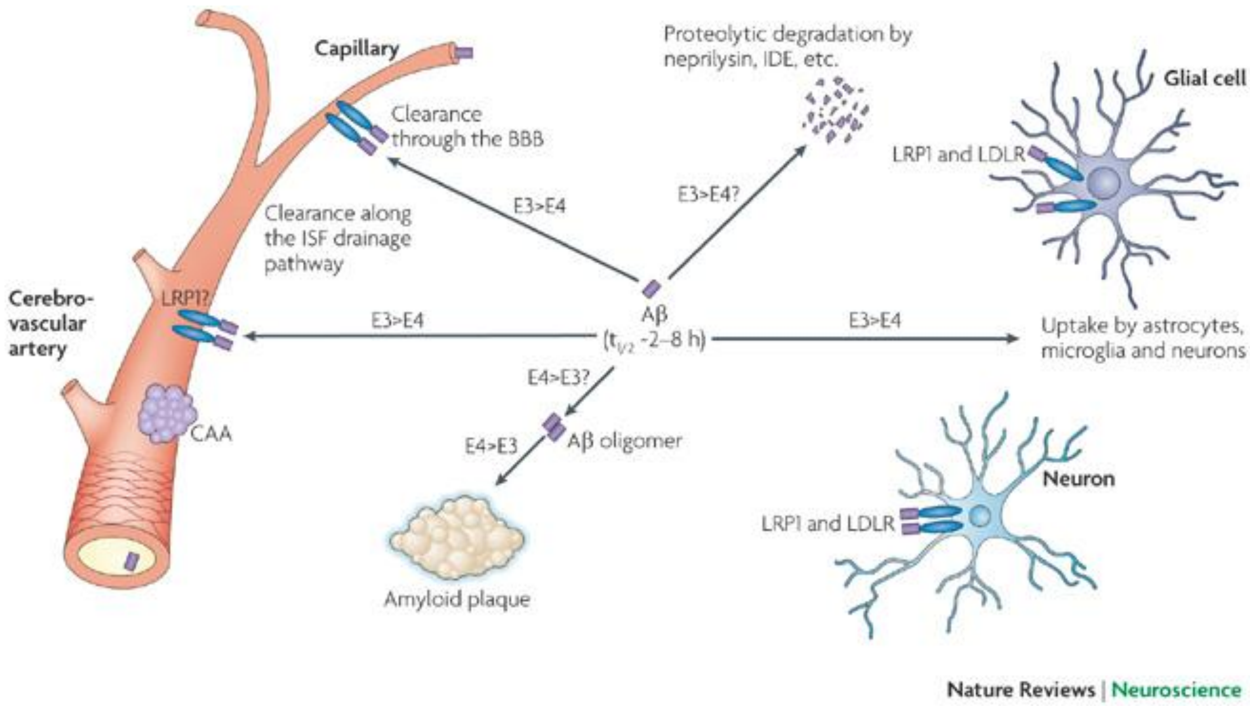

Figure 4 | **Major Aβ clearance pathways in the brain: role of APOE isoforms.** Amyloid-β (Aβ) accumulation in the brain parenchyma leads to the formation of Aβ oligomers and amyloid plaques, which are toxic to neurons. Major Aβ clearance pathways include receptor-mediated clearance by cells in the brain parenchyma (neurons and glia), along the interstitial fluid drainage pathway and through the blood-brain barrier, and proteolytic degradation by endopeptidases. The comparative effects of APOE3 and APOE4 are indicated (Bu 2009).

Neurotoxicity

Aβ oligomers are highly toxic to neurons, inhibiting the viability of neurons in vitro (81), and the memory of animal models (82). ApoE4 augments Aβ oligomer toxicity to a larger extent than apoE3 in cultures (81). It has also been found to act synergistically with Aβ aggregates to induce neurodegeneration in the mouse brain. Neurodegeneration was not observed in mice that had Aβ accumulation due to overproduction induced by mutant APP, only in mice expressing human apoE4 (83).

Tangle formation

Hyperphosphorylated microtubule-associated protein tau (tau) is toxic to neurons, and is the main component of neurofibrillary tangles (57). It is essential for Aβ-induced neuronal dysfunction, as reducing endogenous tau blocks Aβ-induced cognitive impairments in mouse models (84). ApoE4 is normally expressed in neurons only under certain conditions, such as after an injury (85). However, an increased tau phosphorylation in neurons has been observed in uninjured mice expressing human apoE4, suggesting a neuron-specific effect of apoE4 on tau phosphorylation (86). It is possible that in AD brains, abnormal apoE expression in neurons facilitates tau hyperphosphorylation (57).

Cholesterol transport

The primary function of apoE in the brain is to transport cholesterol, mainly from astrocytes to neurons. Cholesterol is an essential component of membranes and myelin sheaths and is crucial for synaptic integrity and neuronal function (87). In the adult brain, there is reduced synthesis of and increased demand for cholesterol in neurons, which makes the need for active cholesterol transport greater. Cholesterol from apoE-lipoprotein particles secreted by astrocytes is essential for the formation of synapses in vitro (FIG 5) (88). Cholesterol levels in AD brains are lower than in healthy brains (89). There is some evidence that apoE4 may be less efficient than apoE3 in transporting brain cholesterol (90), and apoE4 is less efficient than apoE3 in promoting cholesterol efflux from both neurons and astrocytes (91). Using cultured astrocytes from APOE targeted replacement (TR) mice[6], it was shown that fewer apoE3 than apoeE4 molecules are needed to generate lipid particles of a given size, suggesting that apoE3-expressing astrocytes can supply neurons with more cholesterol than apoE4-expressing astrocytes (92).

ApoE also mediates the transport of other brain lipids, such as sulfatide, an oligodendrocyte-synthesized lipid that is crucial for dendritic spine and myelin sheath integrity. Sulfatide is a potential biomarker for AD diagnosis, as its levels are reduced in AD brains (93).

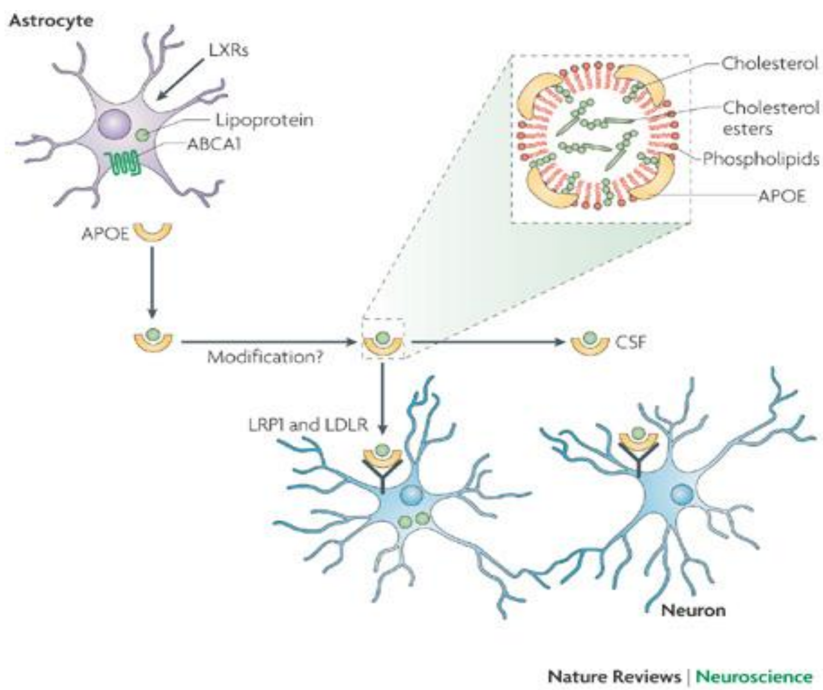

Figure 5 | **Synapse formation and repair depend on cholesterol transport from astrocytes to neurons.** APOE that is secreted by astrocytes assembles cholesterol and other lipids into lipoprotein particles. Cholesterol and other lipids transported to neurons have important roles in synapse formation and repair. The inset shows the main components of APOE-lipoprotein particles: cholesterol, cholesterol esters and phospholipids (Bu 2009).

[6] A model of human APOE expression in which the human APOE gene is inserted into the mouse APOE gene locus. The expression of human APOE in these mice temporally and spatially resembles that of endogenous mouse APOE.

Synaptic plasticity and dendritic spine integrity

Synaptic failure is an early pathological feature of AD (94). ApoE-mediated lipid redistribution and signaling play important parts in synaptic integrity and plasticity. Increasing evidence suggests that apoE isoforms differentially regulate synaptic plasticity and repair. Human APOE4 TR mice display synaptic deficits in the absence of neuropathology (95). Long-term potentiation, i.e. long lasting enhancement of signal transmission between neurons, in the hippocampus of APOE4 TR mice is also significantly impaired, compared to APOE3 TR mice and wild-type mice (96).

ApoE expression in the brain is upregulated after neuronal injury. The primary function of injury-induced apoE expression is probably to redistribute lipids and strengthen apoE-mediated signaling for neuronal and synaptic repair (57). It has been shown in vitro that apoE3 increases neurite outgrowth, whereas apoE4 either decreases outgrowth or has no effect (97). Similarly, apoe3-containing lipoprotein particles are more effective than apoE4 particles in protecting CNS neurons from apoptosis (98). Collectively, these studies indicate that apoE4 is less effective than apoE3 in maintaining and repairing synapses and neurons, which may explain why under certain conditions such as severe head trauma and stroke, individuals with apoE4 tend to have a poorer outcome. It is possible that LOAD is a result of cumulative injury from Aβ and other neuronal stresses, such as oxidation and inflammation, and that APOE4 carriers for this reason are more prone to developing LOAD (57).

The apoE isoforms also differentially regulate dendritic spines during aging. ApoE3-expressing and wild-type mice have a higher density of dendritic spines than apoE4-expressing mice at 2 years of age, but not at 3 weeks of age. This age-dependent difference suggests that the effects of the apoE isoforms on neuronal integrity may relate to the increased risk of dementia in aged individuals that express apoE4. In patients with AD and in aged controls the apoE4 dose inversely correlates with dendritic spine density (99).

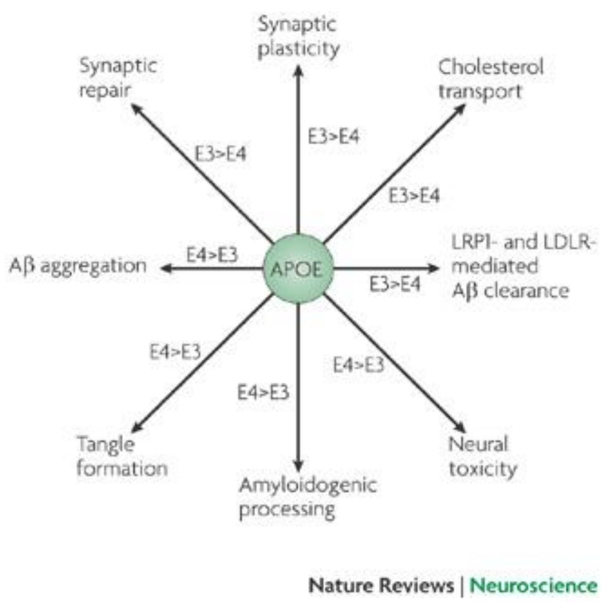

Figure 6 | **Roles of APOE isoforms in the healthy brain and AD pathogenesis.** Summary of APOE functions in normal brain function and the pathogenic processes of AD. Differential regulation by APOE3 and APOE4 are indicated. Aβ, amyloid-β; LDLR, low-density lipoprotein receptor; LRP1, LDLR-related protein 1 (Bu 2009).

Genetic testing

In prognosis

While the effect of the APOE4 allele on the risk and age of onset for AD is generally consistent in most studies, there have been numerous conflicting reports regarding whether the different APOE alleles influence the rate of cognitive decline following dementia onset (56). Craft et al. suggested that homozygous carriers of the APOE4 allele suffered a more rapid cognitive decline and loss of daily function (100), whereas Frisoni et al. found no such association (101). Growdon et al. found that the APOE genotype did not influence the rate of cognitive decline (102), and Kurz et al. stated that carriers and non-carriers of the APOE4 allele did not significantly differ in cognitive functioning and everyday performance over the first three years after being diagnosed with AD (103). Hoyt et al. found that homozygous carriers of the APOE4 allele suffered a slower rate of cognitive decline (104). Collectively, these studies may indicate that the mechanisms which put APOE4 carriers at greater risk for developing AD either precede or play out their influence at an early point in the disease process, or that the biological processes contributing to the onset of AD are different from those determining its clinical course. A study by Cosentino et al. from 2008 concluded that the APOE4 allele may influence the rate of cognitive decline most significantly in the earliest stages of Alzheimer disease (105), more or less in keeping with the above hypothesis. Understanding whether APOE plays a mechanistic role in the progression of AD is an important question to address in the future (56).

In the eyes of the patient

Susceptibility testing for AD, i.e. testing for the APOE4 allele, differs in important ways from predictive testing for disease-causing polymorphisms, such the presenilin-1 and -2 mutations. It is relevant to a much larger at risk population, yet provides much less certain information than predictive testing (106). This limitation, coupled with a general lack of treatment options for AD, has prompted several consensus statements cautioning against susceptibility testing in asymptomatic individuals (107). However, given treatment advances, potential patient demand, and clinical trials seeking at-risk samples, there is a need to examine genetic risk assessment for AD in a research context (108).

The Risk Evaluation and Education for Alzheimer's Disease (REVEAL) study was published in 2005, and assessed the following questions regarding genetic susceptibility testing: Who seeks genetic assessment and why? How do apolipoprotein E results affect risk perception? What psychological impact does genetic risk assessment have? And, how does risk information affect participants' health and insurance behavior? REVEAL was a randomized controlled study, among 162 first-degree relatives of AD patients (109).

All participants were asked to rate the importance of possible reasons for seeking risk assessment. Most important reasons were, in order of importance: to arrange personal affairs; the hope that effective treatment will be developed; to arrange long-term care; to prepare relatives for the possibility of the disease; to do things sooner than planned; and hope of relief when learning of low personal risk. By gauging interest in risk assessment, the REVEAL study found that women, college educated people, and persons under the age of 60 are particularly interested in having their risk assessed (109).

Analyses regarding the comprehension and retention of risk information suggested that participants recalled their APOE genotype better than their lifetime risk estimate given as a percentage. In fact, in a subgroup of women who were all given a lifetime risk estimate of 29 %, those learning they had no APOE4 allele had a greater reduction in anxiety than those given no APOE information. For patients learning that they did have the APOE4 allele, feelings of anxiety were not increased, possibly because they already perceived themselves as being at high risk. These findings highlight the powerful effects that genotype information can have on participants, even when presented as part of a multivariate risk assessment (109).

Regarding health and insurance behavior, participants learning that they had the APOE4 allele were more likely to change their long-term care insurance, compared to APOE4 negative patients. No differences were found on the basis of lifetime risk estimates. After one year, 53 % of APOE4 positive participants reported at least one positive health behavior change (e.g. exercise or diet), while the same was true for only 24 % of APOE4 negative participants and 31 % of controls, indicating that AD risk information in the form of genetic susceptibility testing may motivate engagement in risk reduction activities (109).

Results from the REVEAL study of 2005 suggest that most participants experienced the same or lower levels of anxiety about AD following risk disclosure, and there were no significant posttest group differences within the first year after information was given. In some sub-groups APOE4 negative participants reported lower levels of anxiety. It is important to note that the study put a lot of emphasis on educating its participants on many aspects of the testing and the disease (109). The results of a second REVEAL study were published in 2009. No significant differences in levels of anxiety, depression or test-related distress were found in this study either, but the consensus remains that asymptomatic persons should not be tested for APOE genotype. In both studies people with high depression or anxiety scores were screened out before initiation of testing, and the emotional effect of risk disclosure is still a major concern. In the future, these concerns may be outweighed if APOE genotyping is discovered to predict for instance treatment efficacy or a risk of side effects (110).

In adapting treatment

Current treatment of AD is based on the use of two classes of drugs. Cholinesterase inhibitors are designed to combat impairment of cholinergic neurons by slowing degradation of acetylcholine after its release at synapses. Memantine is meant to prevent overstimulation of a certain subtype of glutamate receptors, which are perceived to contribute to the pathogenesis of AD (111;112). In clinical trials, both cholinesterase inhibitors and memantine have shown beneficial but modest effects on cognitive scores and daily function (113;114), however, their cost effectiveness has been questioned (115). None of these drugs specifically target apoE in any way.

Future therapeutic strategies for AD will most likely involve targeting the main culprits of the disease, such as Aβ and tau (116). Approaches to targeting these substances may involve interacting with the different isoforms of apoE (TABLE 2) (57). As apoE4 is a strong risk factor for AD, whereas apoE3 is not, one attractive approach is to convert apoE4 to an apoE3-like molecule. The domain interaction that exists in apoE4 but not in apoE3 seems to be responsible for most of the apoE4-associated neuropathology, and as such provides a possible target. A few molecules that can disrupt the apoE4 domain interaction have been identified in vitro, but whether they can diminish apoE4's toxic functions in humans is currently unknown (117).

Table 2 | **Strategies of APOE- and APOE receptor-based AD therapy** (Bu 2009).

Strategy	Method	Benefit
Convert APOE4 to APOE3	Disrupt APOE4's domain interaction using a pharmacological approach	Increase APOE3-related function and decrease APOE4-related function
Increase APOE level	LXR agonists using a pharmacological approach	Increase Aβ clearance, lipid homeostasis and synaptic function
Pseudo APOE	APOE-mimetic peptides	Decrease neurotoxicity and inflammation
Block APOE–Aβ interaction	Aβ12–28 using a pharmacological approach	Decrease Aβ aggregation
Block APOE fragmentation	Pharmacological approach	Decrease tau phosphorylation and mitochondria toxicity
Increase APOE lipidation	Increase ABCA1 expression with LXR agonists; other pharmacological approaches	Increase APOE half-life and decrease amyloid deposition
Increase LRP1 and/or LDLR level	Pharmacological approach	Increase Aβ clearance, cholesterol transport and synaptic plasticity
Increase APOER2 and/or VLDLR level	Pharmacological approach	Increase APOE signalling and synaptic plasticity

Aβ, amyloid-β; AD, Alzheimer's disease; APOE, apolipoprotein E; APOER2, APOE receptor 2; LDLR, LDL receptor; LRP1, LDLR-related protein 1; LXR, liver X receptor; VLDLR, VLDL receptor.

Another potential strategy is to regulate apoE expression levels in the brain, perhaps by interacting with promoter polymorphisms of the APOE gene (118). However, this strategy requires careful consideration into whether apoE4 effects in the brain are caused by a loss of protection, a gain of toxicity, or both (57). It has been demonstrated that apoE4 has a faster turnover and lower steady-state concentration in APOE4 TR mice than in APOE3 TR mice, suggesting that low levels of APOE4 may directly contribute to AD pathogenesis, perhaps due to reduced apoE function in lipid metabolism, synaptic repair and Aβ clearance (119). Therefore, increasing the expression of apoE in all APOE genotypes may prevent or slow disease progression. However, increasing the expression of apoE4 in particular, may also generate deleterious effects such as slowing Aβ clearance through the BBB (76) and augmenting Aβ neurotoxicity (120).

There are several other strategies that involve interacting with apoE (Table 1), but they do not differentiate between apoE isoforms. However, strategic combinations of modulations of the Aβ and apoE pathways, whether they pertain to apoE4 or not, remain highly promising avenues of investigation for combating AD (57). As none of these strategies are ready for clinical use, they do not affect the current consensus on predictive genetic testing (116).

A 2009 review by Low et al. systematically reviewed the influence of apoE on the effects of potentially modifiable mid and late life risk factors for dementia, such as cardiovascular factors, lifestyle risk factors, medications, and other risk factors. The reviewers found only a few associations between apoE4, dementia and risk factors. That the detrimental effect of current smoking is limited only to persons without apoE4 was shown by four out of four available studies. Three out of four available studies showed that apoE4 increases the risk of dementia associated with greater fat consumption. Three out of five studies showed that apoE4 increases the protective effect against dementia associated with the use of hormone replacement therapy, while one out of the two non-significant studies suggested a trend. The review found evidence that apoE4 does not modify the risk of dementia associated with measures of, and treatments for cardiovascular disease, other dietary factors, or estradiol levels. For all other risk factors reviewed, there was inconsistent or contradictory evidence. Low et al. concluded that there is insufficient evidence for the recommendation of APOE genotyping to assist with tailoring risk reduction recommendations for dementia (121).

Association with other diseases in the 21st century

The APOE4 allele used to be considered a risk factor for an array of other diseases, including several neurodegenerative ones. Although initially associated with elevated cholesterol-levels and cardiovascular disease, more recent studies have shed some doubt on the assumption that the APOE4 allele in itself is a risk factor. A 2007 review concludes that the effect of APOE4 on cardiovascular disease is dependent on ethnicity, environmental background, and age (122). Recent studies have contradicted previous assumptions that APOE4 is associated with low bone mineral density and osteoporosis, this apparent contradiction may be due to the same set of circumstances as for cardiovascular disease (123-125). HIV-positive homozygous carriers of APOE4 have been shown to exhibit a more rapid disease progression, and especially a more rapid progression to death than homozygous carriers of APOE3. Interestingly, the same was not detected for the progression of HIV-associated dementia, a neurological condition with clinicopathological features similar to those of AD (126). No certain pathogenetically relevant mechanism has been observed linking APOE4 to multiple sclerosis (MS), and as of now we can only assume subtle effects of the apoE isoforms on the progression of MS (127). A 2008 review found that Parkinson's disease dementia (PDD) is not related to APOE4, and concludes that there are different pathological mechanisms that lead to PDD and AD (128). The APOE4 allele has been reported to hasten the progression of diabetic neuropathy (129), but is not a susceptibility gene for idiopathic or diabetic neuropathy (130). Studies conducted on the association between the APOE4 allele and AD in persons with Down syndrome have yielded inconsistent results (131).

The association between APOE4 and cerebral amyloid angiopathy, however, remains clear, as concluded in a 2009 review (132).

CONCLUSION

It is clear that the APOE4 allele is a strong risk factor for LOAD, although the amount it risk it conveys is somewhat dependent on the ethnical and environmental backdrop. For Caucasians, APOE4 increases the risk greatly, in a dose-dependent fashion, putting homozygotes at an even greater risk than heterozygotes. An emerging body of data has identified multiple pathways that could explain the pathogenic nature of APOE4. These include Aβ production and clearance, neurotoxicity, tangle formation, cholesterol homeostasis, and neuronal plasticity and repair. From these, we must hope to identify the pathways most relevant to the pathogenesis of LOAD, and the ones that present opportunities for treating the disease more efficiently than today.

As of today, testing for APOE genotype offers no obvious benefits to patient. It does not present us with any certainty of prognosis, there is no available treatment that differentiates between genotypes, and there is insufficient evidence that APOE testing will assist in risk reduction approaches. However, research does not show any significant detrimental effect on patients' mental health from testing, and, hopefully, as new treatments and risk reduction schemes are developed, APOE genotyping will have its benefits soon enough.

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